POLARIZED AND NON-POLARIZED LEAF REFLECTANCES OF
*COLEUS BLUMEI**

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GRANT L., DAUGHTRY C. S. T. and VANDERBILT V. C. *Polarized and non-polarized leaf reflectances of* Coleus blumei. ENVIRONMENTAL AND EXPERIMENTAL BOTANY **27**, 139–145, 1987.—It is proposed that, through measurement of polarized, reflected radiation, leaf reflectance can be separated into two components: a polarized component from the first surface of the leaf, and a non-polarized, diffuse component primarily from the internal leaf structure. The reflectance of three variegated portions of *Coleus blumei*, Benth. was measured in five wavelength bands of the visible and near-infrared spectrum with a polarization photometer. The polarized and non-polarized components of the reflectance factor and the degree of polarization were calculated. The polarized component was independent of wavelength, demonstrating that the polarized reflectance arose from the surface of the leaf. Differences in the polarized component were presumed to result from variations in surface features. The values of the non-polarized component depended on leaf pigmentation and on wavelength, demonstrating that it emanated from the internal leaf structure. The degree of polarization varied with wavelength and was greatest in regions of the spectrum where absorption occurs.

INTRODUCTION

LEAF reflection studies illustrate the physiological and physical interactions of optical radiation with plant tissue. Information derived from these studies can help in explaining reflection from vegetative canopies and provides a basis for interpretation of photographic and multispectral remotely sensed data from agricultural, forest and other botanical resources of the earth.

Radiation reflected from leaves has two com-

ponents. The first component arises at the leaf surface from radiation scattered or reflected at the air–cuticle interface, the first dielectric discontinuity encountered by the incident beam. Radiation refracted into the leaf at this discontinuity may be scattered many times as it passes through the heterogeneous plant tissue. The second component consists of the radiation that emerges from the bulk of the plant tissue on the side of the leaf toward the illumination source. The spectral radiant flux scattered by the surface

and by the volume of the leaf are determined by the compositional and geometric characteristics of the leaf's anatomy.[2]

Most studies of leaf reflection measure the reflected radiation without attempting to separate it into its component parts. These studies rely on measurements taken with the Ubrecht integrating sphere, an instrument which measures all radiation as though it were diffuse (non-directional). In interpreting the results of these studies, investigators assume the reflection from the leaf to be Lambertian (reflecting uniformly in all directions), and/or assume reflection from the first surface to be insignificantly small.[6] Thus the interpretation of the reflection is solely in terms of the effect of the internal structure, i.e. arrangement of cells, distribution of chloroplasts, water content.

The few studies of first surface reflection refute the assumption that this is small and contend that it may be a sizeable fraction of the total reflected radiation.[9,10,13] Thus in measuring only total leaf reflectance and implicitly assuming it is dependent solely on internal leaf structure, investigators may have missed some information about the characteristics of the surface of plant leaves.

The first surface reflection is determined by characteristics of the plant cuticle. The outermost layer of the cuticle consists of epicuticular wax which may be amorphous, semi-crystalline or crystalline, depending on its chemical composition. The chemical composition of the wax is genetically determined and may vary with species, ontogenic development and environmental conditions.[1] The structure of the wax determines the surface roughness and thus the scattering mechanisms of the first surface reflection.

Small wax particles and facets of wax particles with dimensions less than the wavelength of incident radiation preferentially scatter shorter wavelengths. The small particles and facets, if sparsely distributed on the surface, act as Rayleigh scatterers. The radiation scattered by a Rayleigh-sized particle will be completely polarized at 90° to the incident beam, partially polarized at other angles, randomly directed and enhanced in the blue due to preferential scattering of small wavelengths. However, most often this scattered blue flux is non-polarized, indicating the scatterers,

while small, are too densely distributed to satisfy the criteria for Rayleigh scattering.

Wax particles and facets of wax particles with dimensions equal to or slightly larger than the wavelengths of incident radiation may also scatter radiation. The radiation scattered by these particles tends to be partially polarized, is randomly directed and appears white.

Wax particles, facets of wax particles, and areas of amorphous wax with dimensions greater than the wavelengths of incident radiation are optically smooth and will reflect radiation specularly. The angle of reflection equals the angle of incidence. The specularly reflected radiation is completely polarized at the Brewster angle (55°, assuming a refractive index of 1.43 for the cuticle),[12] partially polarized at other angles, and appears white.

Radiation reflected from the bulk of the leaf tissue is multiply scattered at each dielectric discontinuity encountered as light travels through the tissue. The degree to which the radiation is scattered depends on the optical dimensions, the values of the indices of refraction, the angles formed by the rays of radiation and the refractive surfaces, and the presence or absence of absorbing substances. The radiation emerging from the bulk of the leaf will be non-preferentially directed (diffuse) due to the multiple scattering,[7] will possess a wavelength dependency determined by pigments and other absorbing substances within the leaf, and will be non-polarized.

This study was devised to test the hypothesis that, through measurement of polarized, reflected radiation, leaf reflection can be separated into two components: a polarized reflection from the first surface of the leaf and a non-polarized, diffuse reflection primarily from the internal leaf structure. Once these two components, originating from physically separable entities, are identifiable, they may be considered as independent sources of information about the leaf.

MATERIALS AND METHODS

Fully expanded leaves from two variegated hybrids of *Coleus blumei*, Benth. were used in this study. The leaves of one hybrid were white in the center of the lamina surrounded by green margins. These leaves provided samples of green

and white pigmentation. The other hybrid's leaves were reddish-purple in the center of the lamina surrounded by green margins. These leaves provided samples of the reddish purple pigmentation. Measurements were taken with a portable polarization photometer[11] which allows non-destructive measurements of leaf reflection at the Brewster angle (55° from normal). This instrument has a self-contained irradiance source which illuminates the sample with a non-polarized, collimated beam at 55° from normal. A filter wheel containing interference filters controls the spectral properties of illumination. The wavelength bands include four wavelengths in the visible region of the spectrum, centered at 450, 500, 550 and 650 nm, and a near-infrared band centered at 730 nm (half power band width of 70 nm). A polarization analyzer and photodetector are positioned to measure reflectance at 55° from normal (phase angle equals 110°).

The amount of radiation, represented as a voltage, reflected by a sample, V_{sample}, was calibrated using measurements of (1) a painted $BaSO_4$ standard, V_{BaSO_4} of known reflectance,[4] R_{BaSO_4}, and (2) the dark level of the instrument, V_{dark}. The bidirectional reflectance factor, R,[8] of the sample is the amount of flux received from a sample surface divided by the amount of flux which would be received from an ideal, hypothetical, perfectly diffuse, perfectly white standard. Both surfaces must be similarly viewed and similarly illuminated; illumination must be well-collimated and the size of the solid angle of the viewing cone must be small. The R of the sample is then:

$$R = [(V_{sample} - V_{dark})/(V_{BaSO_4} - V_{dark}] * R_{BaSO_4} * 100$$

Each observation consisted of a pair of measured reflectance factors, R_{max} and R_{min}, representing, respectively, the maximum and minimum amount of radiation transmitted by the polarization analyzer. From these calculated values the following variables were determined:

$$R = (R_{max} + R_{min})/2.0$$
$$R_q = (R_{max} - R_{min})/2.0$$
$$R_n = R - R_q$$
$$P = (R_q/R) * 100.$$

R is the bidirectional reflectance factor of the leaf

and is equivalent to the value measured at the same angle but without the polarization analyzer. R_q represents the polarized component of the reflectance factor. R_n represents the non-polarized component of the reflectance factor. P is the degree of linear polarization and is the ratio of the polarized component of the reflectance factor of the leaf to the reflectance factor of the leaf.

Two observations, on both the adaxial and abaxial sides of each of six leaf samples of the three variegated pigments, were acquired *in situ*. Statistical differences due to pigmentation, wavelength and side of the leaf were tested using analysis of variance.

RESULTS AND DISCUSSION

Figure 1 illustrates the reflectance factor, R, as a function of pigmentation and wavelength. The results agree with the laboratory findings of HOFFER and JOHANNSEN[5] who measured hemispherical reflectance of coleus with an integrating sphere spectrophotometer. The results (Fig. 1) show the reflectance of a leaf to be essentially dependent on leaf pigmentation in the visible portion of the spectrum. The reflectance factor of the green portion of the leaf variegation (Fig. 1a and d) has low value in the blue (450 nm), blue-green (500 nm) and red (650 nm), and high value in the green (550 nm) and near-infrared (730 nm) wavelength regions. This reflectance curve is characteristic of green leaves where chlorophyll is the dominant pigment and gives a mirror image of the absorption spectrum of chlorophyll with absorption bands in the blue and red regions of the spectrum and low absorption in the green.

The reflectance factor of the reddish purple portion of the variegation (Fig. 1b and e) is low throughout the visible region. Both chlorophyll and anthrocyanin are dominant pigments in this tissue. The generally low reflectance in the visible results from absorption by these pigments and illustrates the effectiveness of chlorophyll, in concert with other pigments, in harvesting radiation from the range of the visible, high energy region of the spectrum.

The reflectance factor of the white portion of the leaf (Fig. 1c and f) is high throughout the visible region. There is some variation with wave-
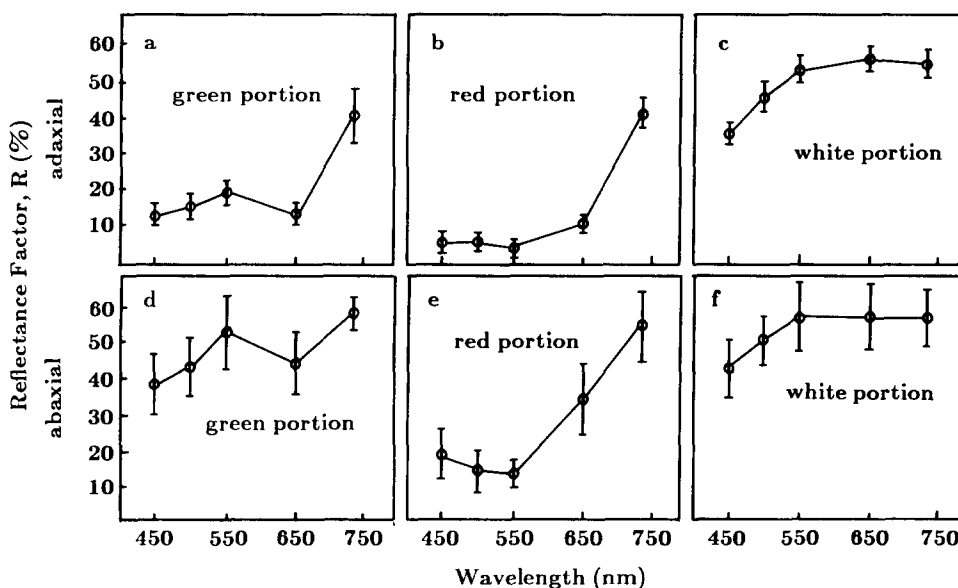
Fig. 1. Reflectance factor, R, as a function of wavelength of *Coleus blumei*. Each datum represents the mean ± one standard deviation of 12 observations.

length. The reflectance factor in the blue and blue-green bands is lower than that in the green and red bands. This suggests that although the white tissue appears to be void of pigments it does contain some carotenoids which absorb in the blue and blue-green regions of the spectrum.

For all leaves, regardless of their primary pigment, the reflectance factor is greatest in the near infrared band. Reflectance in this region is presumed to be governed by a lack of pigment absorption and by cellular structure.[2,6] The fact that all three pigments show the same near-infrared reflectance suggests that there is no significant difference in the structure of the plant tissue containing these diverse pigments.

Reflectance factors from the abaxial side (Fig. 1d–f) are greater than those of the adaxial side (Fig. 1a–c) for all plant samples. This greater reflectance is attributable to the dorsiventral structure of the coleus leaves. Spongy mesophyll with its irregular arrangement of cells close to the abaxial surface contributes more to the multiple scattering of radiation than the palisade parenchyma cells close to the adaxial surface.[3]

The non-polarized component of the reflectance factor, R_n (Fig. 2), exhibits the same wavelength dependency as does the reflectance factor, though naturally lower in value. The wavelength dependency of the non-polarized reflectance on the dominant pigment of the leaf variegation suggests that most, if not all, of the radiation represented by the non-polarized component emanates from the interior, bulk of the leaf tissue, though there may be some small contribution from particle scattering at the leaf surface. Visible radiation moving through the leaf tissue is attenuated by the presence of plant pigments. Light absorbed by pigments is removed from the radiation multiply scattered within the tissue, thus reflectance from the interior of the leaf is low at wavelengths of absorption and great at wavelengths where there is little or no absorption. This is clearly demonstrated in the non-polarized component. In the green variegation, the dominant pigment chlorophyll absorbs in the blue and red where reflectance is low (Fig. 2a and d). Absorption is least around 550 nm where the reflectance peaks. When anthocyanin is added to the pigment
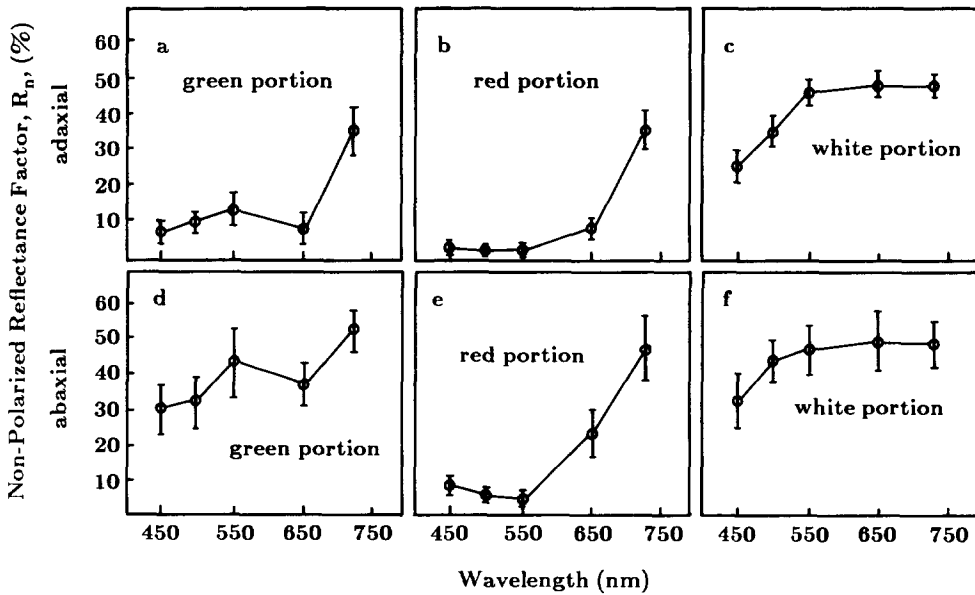
FIG. 2. Non-polarized reflectance factor, R_n, as a function of wavelength of *Coleus blumei*. Each datum represents the mean $\pm$ one standard deviation of 12 observations.

system, as in the reddish purple variegation, the reflectance is low throughout the visible region of the spectrum (Fig. 2b and e). The presence of carotenoids which absorb in the blue and blue-green results in low reflectance at these wavelengths and high reflectance in the green and red (Fig. 2c and f).

In contrast with the non-polarized component, the polarized component of the reflectance factor, R_q, has no wavelength dependency (Fig. 3). This lack of wavelength dependency clearly demonstrates that the polarized component is a first surface phenomenon. The polarized radiation never entered the leaf tissue to be attenuated by pigment absorption. There is no enhancement of the blue band in the polarized component of the reflectance factor from these coleus leaves. Thus small particle scattering from the surface of the leaf is insignificant. The features of the coleus leaf surfaces responsible for this polarized, first surface scattering must be of dimensions greater than the wavelengths of visible radiation.

The polarized component measured from the abaxial side of the leaves is the same for all three pigmentation samples (Fig. 3d–f), suggesting that the adaxial surfaces are also similar for all three samples. Leaves with white variegation have equal polarized reflectance factors from both the adaxial and abaxial surfaces, whereas leaves with green or reddish purple variegation have greater polarized reflectances from the abaxial surface than from the adaxial surfaces. Any differences in polarized reflectances suggest that the features of the surface differ, depending on either hybrid or position on the lamina.

When the polarized component (Fig. 3) is considered as a fraction of the reflectance factor (Fig. 1) across the spectrum (degree of polarization, Fig. 4) the relative contribution of the polarized, surface reflectance is substantial. In regions of the spectrum where absorption by leaf pigments is high and reflectance is low, the polarized component of reflectance can account for more than half the reflected radiation. Factors which affect the surface of the leaf, its cuticle and outermost wax layer should be manifest in the leaf reflectance and perhaps apparent in regions of high absorption and low reflectance. Measure-
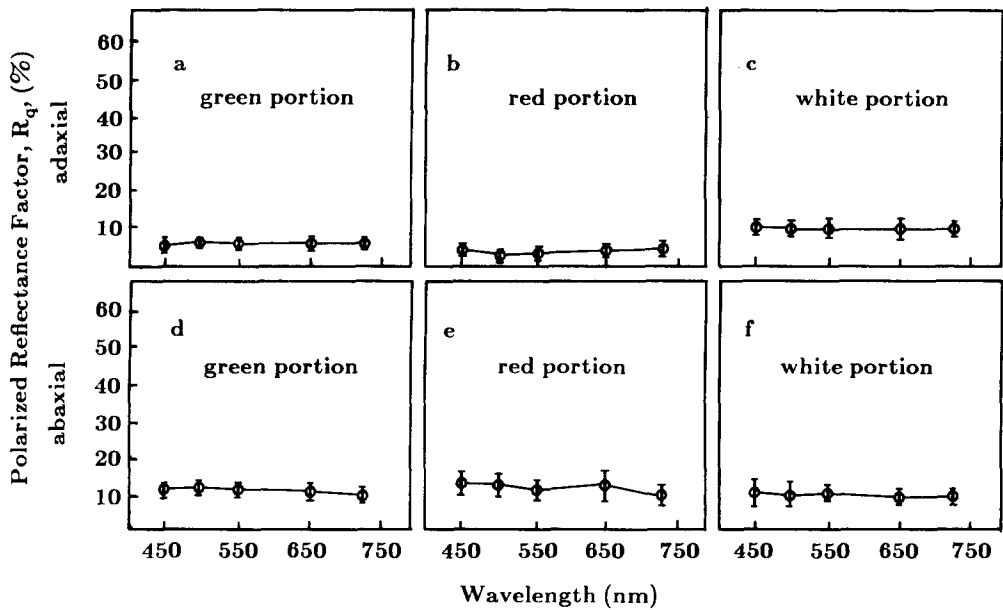
FIG. 3. Polarized reflectance factor, R_p, as a function of wavelength of *Coleus blumei*. Each datum represents the mean ± one standard deviation of 12 observations.

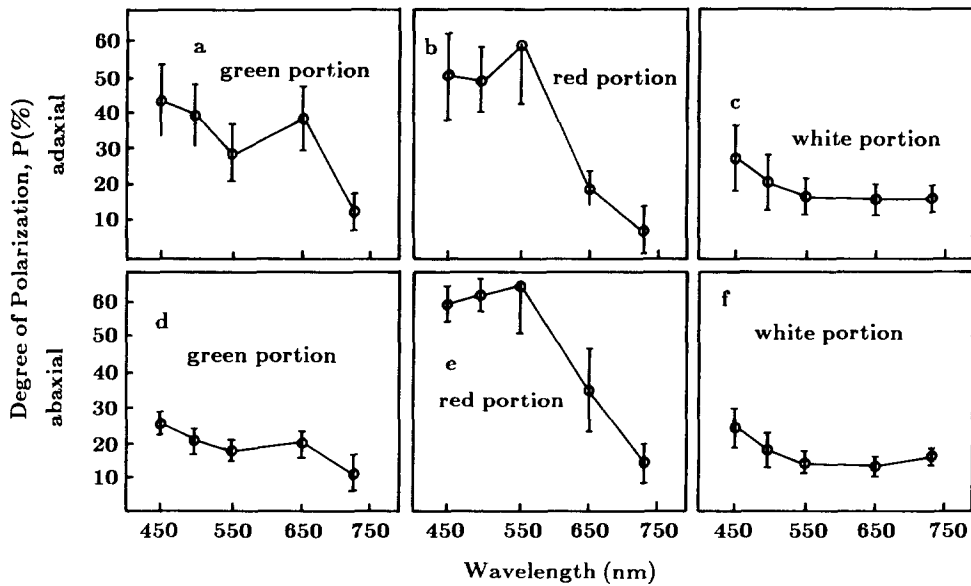
FIG. 4. Degree of polarization, P, as a function of wavelength of *Coleus blumei*. Each datum represents the mean ± one standard deviation of 12 observations.

ment of the polarized component of reflectance should allow examination of these effects.

It is important to recognize that these measurements of the polarized component of reflectance are taken at the Brewster angle and radiation polarized through specular reflectance is completely, linearly polarized at this angle. At angles less than the Brewster angle, the specularly reflected radiation will be less and only partially polarized, so the polarized reflectance will be less. At angles greater than the Brewster angle the specularly reflected radiation should increase, but it will be only partially polarized, thus the polarized component of reflectance measured at angles greater than the Brewster angle may not fully represent the increase in reflectance due to increasing angles.

CONCLUSIONS

Radiation reflected from a leaf can be divided into two components: one from the surface of the leaf and one primarily from the bulk of the leaf tissue, through measurement of the polarized reflectance at the Brewster angle. Differences in the polarized reflectance observed for the three pigmented samples suggest that information about the surface is contained in the polarized component of reflectance. The fact that the non-polarized component of reflectance and the total reflectance have similar wavelength dependencies is significant and supports the accepted interpretation of reflectance measurements, that internal reflection predominates. Consideration must however be given to the surface reflectance and to factors which affect the surface of the leaf.

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